

Central-East Regional Biomass Research Center Accomplishments 2010 to 2014

Accomplishments: The list below summarizes the research accomplishments of the Central-East Regional Biomass Research Center related to the development of perennial grasses for dedicated bioenergy feedstocks between 2010 and 2014. The accomplishments are summarized by ARS location.

Grain, Forage, and Bioenergy Research Unit, Lincoln, NE (NP215)

Project Title: Improving bioenergy and forage plants and production systems for the central U.S.
Project Nos: 5440-21000-030-00D and 5440-21220-027-00D

Switchgrass grown for biomass energy results in significant soil carbon (C) sequestration.

A long-term switchgrass soil C sequestration study was established in eastern Nebraska in 1998 by ARS scientists at Lincoln, NE and Ft. Collins, CO. The study includes two switchgrass cultivars, three nitrogen (N) fertilizer rates and two harvest treatments. In the 9-year period from the spring of 1998 to the spring of 2007, soil C increased at rate of 0.9 U.S. tons/acre per year in plots in which best management practices were used. Biomass yields and C sequestration was significantly greater in plots in which N fertilizer was used than in plots where no fertilizer was applied. These results fully support switchgrass soil C sequestration data previously obtained in a 5-year study on 10 farms in NE, SD, and ND. In the on-farm study conducted by ARS scientists at Lincoln, NE and Mandan, ND, additional soil analyses has shown that switchgrass biomass production and harvest resulted in very small changes in available soil phosphorus (P). Soil P decreased by only 1.3 lbs per acre of available P per year.

Herbicides used for establishing switchgrass in the mid-continental USA improve establishment success and accelerate biomass production for bioenergy. Weeds limit switchgrass establishment from seed, but few herbicides are labeled for switchgrass establishment. Selected herbicides were tested on stand establishment and subsequent yields of adapted upland switchgrass cultivars in Nebraska, South Dakota, and North Dakota as well as lowland ecotypes in Nebraska by ARS scientists at Lincoln, NE and Mandan, ND. Applying quinclorac plus atrazine resulted in acceptable stands and high yields for all locations and ecotypes. Quinclorac and atrazine is an excellent combination for establishing switchgrass in the mid-continental USA. With good management including the use of herbicides, switchgrass can produce yields equivalent to half of full production the establishment year and can be at full production the year after planting. This research contributed to the labeling of quinclorac for establishing switchgrass for bioenergy in this region and is available for use by farmers.

Improved real-time assay for plant O-methyltransferases. Plant O-methyltransferases are key enzymes in plant metabolism and play a crucial role in the generation of intermediates during lignin biosynthesis. Earlier assays for these enzymes, and ones specifically involved in lignin biosynthesis were cumbersome and/or involved the use of radioactive substrates. A new method developed by ARS scientists at Lincoln, NE relies on fluorescence as a means to detect and quantify the activity of these enzymes. This new assay can be used in genetic studies to modify lignin composition of biomass which can affect its conversion to liquid fuels.

Improved switchgrass seed quality tests improve establishment and initial biomass yields.

The economic viability of growing switchgrass for bioenergy hinges on successful stand establishment during the seeding year. ARS-Lincoln scientists developed an innovative seed lot evaluation test that is based on the number of emerged seedlings per gram of seed in a stress test rather than the percentage of seeds that germinate in a germination cabinet. Using this new test instead of the conventional Pure Live Seed method to determine planting rates resulted in significantly better switchgrass stands and greater biomass yields the first harvest year. Basing switchgrass seeding rates on emerged seedlings per gram with an associated stress test will reduce the risks of failure during the stand establishment year due to poor seed quality and will improve biomass yields for the initial harvests.

Field and year-to-year variation in switchgrass biomass quality and its effects on ethanol yields per ton and production per acre were quantified.

Theoretical ethanol yields were determined from biomass harvested from 10 farms in Nebraska and South and North Dakota for a five year period. Near Infrared Reflectance Spectroscopy (NIRS) calibrations developed by a team of ARS scientists from Lincoln, NE, St. Paul, MN, Peoria, IL, and Madison, WI were used to determine composition and predict ethanol yields. Theoretical ethanol yield varied by year and field, with 5 year means ranging from 91 to 103 gallons per ton of biomass. Total theoretical ethanol production ranged from 187 to 394 gallons per acre across fields planted to forage type switchgrass cultivars. Because of the differences in potential liquid fuel yields per ton, cellulosic biorefineries will need to assess switchgrass quality using a suitable technique such as the NIRS calibrations developed by ARS. Cellulosic biorefineries will need to consider the yearly variation that can occur in biomass production in a region in their business plans.

Long-term switchgrass soil carbon sequestration study indicates assumptions used in previous bioenergy net benefits modeling are erroneous.

The changes in soil organic carbon during the first nine years of a long-term switchgrass and corn soil C sequestration study indicate that all soil C changes were positive and that nitrogen fertility rates and harvest management affected the net increase in soil carbon. ARS scientists at Lincoln, NE and Ft. Collins, CO demonstrated that both switchgrass and corn sequestered soil organic carbon (SOC) down to a depth of 5 feet and over 50% of the soil organic C was sequestered below the one foot depth which is the soil depth most previous modeling work is based. Both switchgrass and corn sequestered 0.9 tons of C per year with the best management practices. The results demonstrate that previous modeling work on the net benefits of bioenergy crops which were conducted assuming uniform responses to management and a shallow one foot soil sampling depth for soil carbon are likely erroneous.

Improved knowledge of autumn dormancy and spring-greening of perennial grasses.

Molecular mechanisms controlling winter-hardiness and survival could be exploited to accelerate improvements of lowland switchgrass cultivars if they were clearly understood. ARS scientists at Lincoln, NE have developed the first insights into the metabolism of crown and rhizome tissues obtained from a winter hardy upland cultivar of switchgrass. Over 30,000 new DNA sequences coding for several genes that likely have an important role in winter-hardiness were obtained. Large datasets of DNA sequences should permit the development of molecular markers for genes

controlling winter hardiness in switchgrass which would greatly facilitate the breeding progress for this economically important trait.

Calibrations for switchgrass biomass composition. Near infrared reflectance spectrometry (NIRS) calibrations for switchgrass biomass composition, developed cooperatively by ARS scientists at Lincoln, NE, Peoria, IL, St. Paul, MN, and Madison, WI, were transferred to the NIRS Forage and Feed Testing Consortium (NIRSC) which is an association of commercial, university, and government research laboratories, plant research companies, and instrument companies that collaborate to improve NIRS analyses methodology. The NIRSC has transferred these calibration sets and associated standard samples to 19 laboratories. The switchgrass NIRS calibrations enables commercial, industrial, academic, and government laboratories to rapidly determine 20 compositional components of switchgrass biomass. The sample cost using the ARS developed NIRS calibrations is approximately \$5 per sample. Conventional analyses methods would cost over \$300 per sample. This technology will significantly facilitate the breeding and management research to develop perennial grasses into bioenergy crops.

Improved bioenergy-type switchgrass cultivar with high biomass yield tested and increased for use in the northern half of the USA. Switchgrass cultivars for the northern half of the US have been limited to upland ecotype switchgrass cultivars because available lowland cultivars have poor winter survival in the region. Lowland switchgrass cultivars have the potential to produce greater biomass yields if they had better winter survival. A new lowland type switchgrass cultivar ‘Liberty’ was released in 2013. Liberty which was developed by ARS researchers at Lincoln, NE by crossing northern upland and southern lowland plants followed by three generations of breeding for winter survival, high biomass yield, and low stem lignin concentration. Over a three year period in trials in NE, WI, and IL, Liberty had excellent winter survival and in eastern Nebraska and northern Illinois had biomass yields that were 2 tons per acre greater than the best available released upland cultivars. Liberty is the first bioenergy type cultivar for the Midwest and the northern Great Plains and will likely be used in the Northeast states. When processed in a biorefinery, the increased biomass yield will result in an additional 160 gallons of ethanol per acre which could fuel an economy car for 5,000 miles.

Identified a total of 342 class III peroxidase genes in the switchgrass genome. Work with other grasses have shown that increases in the levels of specific class III peroxidases are associated with improved resistance to herbivory by piercing-sucking insects. Such information is unavailable for switchgrass. In this work performed by ARS researchers at Lincoln, NE, in collaboration with University of Nebraska scientists, the presence or absence of all the class III peroxidases was documented in switchgrass tissues at different stages of plant development. Using these data, it is now possible to identify specific switchgrass peroxidase genes that are involved in the plant’s response to insect herbivory. Data obtained by these new methods can be used by plant breeders and other researchers to develop switchgrass strains with improved resistance to piercing-sucking insects.

Release of elite lines incorporating brown midrib genes has provided materials for production of commercial hybrids. Two genes, *brown midrib (bmr) 6* and *bmr12*, were incorporated into elite germplasm, alone or in combination, in order to determine effects on lignin precursors, cellulosic bioenergy potential, and utility in new agricultural applications. The

resulting ARS-released lines have served as the basis for scientific research on *bmr6* and *bmr12* throughout the world. Research results have demonstrated the ability of hybrid vigor to overcome decreased yield, shown reduced disease incidence to be associated with these genes, and shown higher ethanol conversion and conversion efficiencies to be associated with these genes. This information has thereby supported commercial efforts to develop a market for proprietary hybrids. Research based on these materials has also identified changes in lignin chemistry and the biochemical and molecular changes responsible, leading to identification of targets for improvement of other bioenergy crops. Since the first release of these materials in 2005, over 1,200 seed packets from these lines have been distributed without charge and without restrictions on their use, to private companies, universities, and national labs on six continents. They have also provided impetus for current research on discovery and description of new *brown midrib* genes, which promises to unlock even more potential for utilization of sorghum and related crops for bioenergy purposes.

Near-isogenic lines containing the *waxy* mutation and adapted to the northern sorghum-growing region of the USA have greater digestibility for conversion efficiency to ethanol.

The existence of multiple alleles for the ‘waxy’ (low amylose grain) trait in sorghum was previously discovered by this project, but the effect of these alleles on grain utility and their distribution in sorghum germplasm was unknown. Four new lines containing a new allele, wx^b , were identified. Energy requirements for gelatinization of waxy genotypes, an indication of digestibility, were generally lower than for non-waxy genotypes, but considerable variation was shown to exist. When grain from *waxy* and WT lines was screened for fungal pathogens, which can affect grain yield or quality, the results showed that *waxy* sorghum lines were not more susceptible to grain infections than wild-type lines. A set of waxy/wild-type isolines, R-lines, A/B-lines, and another set of isolines with wx^a and wx^b alleles have been developed. In addition, the mutations responsible for the waxy phenotype in wx^a and wx^b were identified and molecular markers were developed for both alleles to aid breeding efforts. These sets of lines will be valuable germplasm for enhancing sorghum grain for conversion to bioenergy.

Identification and characterization of three *Bmr* genes and their corresponding proteins.

Bmr6 was cloned and shown to be an enzyme in monolignol biosynthesis, cinnamyl alcohol dehydrogenase (CAD). Similarly, *Bmr2* was cloned and shown to encode another enzyme in monolignol biosynthesis, 4-coumarate Coenzyme A ligase (4CL). The effects of a series of new *bmr12* alleles were characterized on plant tissue and the monolignol biosynthetic enzyme caffeic O-methyltransferase (COMT) encoded by *Bmr12*. These studies linked phenotypes, mutations, protein levels and enzymatic activity together to provide a complete picture of these *bmr* lines. This information allows plant breeders to develop strategies to modify lignin content and its composition in sorghum, and has implications for lignin modification in other bioenergy grasses.

The *brown midrib* genes *bmr6* and *bmr12* are associated with resistance to some fungal pathogens. The incidences of two fungi that commonly infect sorghum grain were significantly reduced in *bmr12* grain. Inoculation of stalks of wild-type and near-isogenic *bmr6* and *bmr12* plants with sorghum pathogens resulted in infected areas on *bmr6* or *bmr12* plants that were the same size or smaller than those on wild-type plants. Therefore, enhancing sorghum for bioenergy and livestock feed using *bmr6* and *bmr12* does not increase disease susceptibility and, for some pathogens, results in increased resistance.

Determined the molecular structure of a sorghum lignin biosynthesis enzyme.

Understanding lignin synthesis is critically important for developing plants with altered biomass composition to be used with emerging bioenergy conversion technologies to produce liquid fuels. The sorghum enzyme hydroxycinnamoyltransferase (SbHCT) is a key enzyme that participates in an early step of lignin synthesis. The structure of this enzyme was determined to understand how the enzyme functions in lignin synthesis. The structure of SbHCT was similar to the structure of other enzymes found in plants. The observations of ARS scientists from Lincoln NE and collaborators explain how SbHCT and other enzymes that share similar structural features can participate in different biochemical pathways in different plant species. Knowledge of this protein structure will enable future research on modifying lignin content and composition of sorghum and other crops for bioenergy.

Developed and evaluated sweet sorghum hybrids. Sweet sorghum has received substantial attention as a bioenergy crop throughout the world, because its processing would be similar to sugarcane. To generate such large quantities of seed, the commercial seed industry will need to produce hybrid varieties on dwarf seed-parent lines, which produce high numbers of seed and are amenable to mechanical harvest. The ability to produce sweet sorghum hybrids using existing dwarf seed-parent lines was evaluated for all traits directly contributing to total ethanol yield. Results of this study demonstrate that hybrid sweet sorghum with performance criteria equivalent to existing sweet sorghum cultivars can be produced on existing seed-parent lines.

Funded Bioenergy Grants:

1. 2009-2013: “The hunt for green every April: Factors affecting fitness in switchgrass”. DOE-USDA Feedstocks Genomics Program. US Department of Energy Grant Number DE-AI02-09ER64829. PI G. Sarath. \$1,181,866.
2. 2011-2016: “Mitigating insect herbivory of warm-season bioenergy grasses - getting ahead of the curve”. USDA-NIFA Competitive Grants Program. NIFA Award Number: 2011-67009-30096. PI G. Sarath. \$997,741.
3. 2011-2012: “Micro/nanomechanical studies of switchgrass composition and cellulose breakdown kinetics” funded through the UNL-Energy Sciences Research, Co-PI G. Sarath. \$100,000.
4. 2007-2011: DE-FG02-07ER64458 BER-U.S. Department of Energy Grant, Genetic Dissection of Bioenergy Traits in Sorghum. PI: W. Vermerris Co-PIs S. Sattler and J. Pedersen. \$750,000.
5. 2011-2016: 2011-67009-30026 USDA-NIFA AFRI Sustainable Bioenergy Grant (2011-2016) The impacts of lignin modification on fungal pathogen and insect interactions in sorghum for cellulosic and thermal bioenergy. PI S. Sattler. \$973,128.
6. 2014-2017: “Heterosis, Drought and Mineral Composition in Switchgrass”. DOE-USDA Feedstocks Genomics Program. PI G. Sarath. \$ 1,173,924. (Pending)
7. 2014-2017: “Exploiting natural diversity to identify alleles and mechanisms of cold adaption in switchgrass”. DOE-USDA Feedstocks Genomics Program. CoPI G. Sarath. \$184,000 (Pending)
8. 2009-2104: PI: Enhancement of USDA-ARS, DOE, and Sun Grant Universities Cooperative Interdisciplinary Research, DOE Sun Grant Regional Feedstock Partnership; USDA-ARS ADODR, PI R. Mitchell \$65,000.
9. 2011-2014: Sustainable production and distribution of bioenergy for the central USA, USDA-NIFA AFRI-CAP, 2011-68005-30411; USDA-ARS Coordinator & ADODR, Leader of

Objectives 1-4, and Co-Director of Objective 2, Sustainable Feedstock Production Systems, Co-PI R. Mitchell \$1,573,499 to location.

10. 2013-2015: Soil and environmental responses to dedicated bioenergy crops on marginally productive croplands, North Central Sun Grant Center; Co-PI R. Mitchell \$94,619.

Selected Publications (2010-2014):

1. Amaradasa BS, Donze T, Heng-Moss T, Sarath G, and Amundsen K (2014) Characterizing differential gene expression in polyploid grasses lacking a reference transcriptome. *OA Biotechnology*. Jan 10:3:1.
2. Bowman MJ, Dien BS, O'Bryan PJ, Sarath G, Cotta MA (2011) Selective chemical depolymerization of switchgrass (*Panicum virgatum* L) xylan with oligosaccharide product analysis by mass spectrometry. *Rapid Comm Mass Spect.* 25: 941-950
3. Bowman MJ, Dien BS, Hector RE, Sarath G, Cotta MA (2011) Liquid chromatography-mass spectrometry investigation of enzyme-resistant xylooligosaccharide structures of switchgrass associated with ammonia pretreatment, enzymatic saccharification, and fermentation. *Biores Technol.* 110: 437-447.
4. Bowman MJ, Dien BS, O'Bryan PJ, Sarath G, Cotta MA (2012) Comparative analysis of end point enzymatic digests of arabino-xylan isolated from switchgrass (*Panicum virgatum* L.) of varying maturities using LC-MSⁿ. *Metabolites* 2: 959-982. Log. No. 286240
5. Dien BS, Miller DJ, Hector RE, Dixon RA, Chen F, McCaslin M, Reisen P, Sarath G (2011) Enhancing alfalfa conversion efficiencies for production of sugars and ethanol by altering lignin composition. *Biores. Technol.* 102: 6479-6486.
6. Dien BS, O'Byran PJ, Hector R, Iten LB, Mitchell RB, Sarath G, Vogel KP, Cotta MA (2013) Conversion of switchgrass to ethanol using dilute ammonium hydroxide pretreatment: Influence of ecotype and harvest maturity. *Environmental Technology* 13-14: 1837-1848.
7. Dowd PJ, Sarath G, Mitchell RB, Saathoff AJ, Vogel KP (2012) Retention of insect resistance in some switchgrass lines with reduced lignin levels. *Genet Resour Crop Evol.* 60: 975-984. Log No. 270782.
8. Follett, R.F., K.P. Vogel, G. Varvel, Mitchell, R.B., and J. Kimble. Soil carbon sequestration by maize and switchgrass grown as bioenergy crops. *Bioenergy Research.* 5:866-875. 2012. Log # 268806
9. Funnell-Harris, D. L., Pedersen, J. F., Sattler, S. E. 2010. Alteration in lignin biosynthesis restricts growth of *Fusarium* species in brown midrib sorghum. *Phytopath.* 100:671-681.
10. Funnell-Harris, D. L. and Pedersen, J. F. 2011. Presence of *Fusarium* spp. in air and soil associated with sorghum fields. *Plant Disease* 95: 648-656.
11. Griess, J. K., Mason, S. C., Jackson, D. S., Galusha, T. D., Pedersen, J. F., and Yaseen, M. 2011. Environment and hybrid influences on rapid-visco-analysis flour properties of food-grade grain sorghum. *Crop Science* 51: 1757-1766.
12. Heng-Moss T, Bradshaw J, Koch KG, Prochaska TJ, Donze-Reiner T, Sarath G (2014) Grow them and we will come to feast –a viewpoint. *BioFPR* 8: 145-146.
13. Jung H-J, Samac DA, Sarath G (2011) Modifying crops to increase the digestibility of cell wall material. *Plant Sci.* 185: 65-77. Invited review.
14. Kiniry, J.R., M.V. Johnson, R. Mitchell, K.P. Vogel, J. Kaiser, S.B. Bruckerhoff, and R.L. Cordsiemon. Switchgrass leaf area index and light extinction coefficients. *Agronomy Journal* 103:119-122. 2011.

15. Koch KG, Fithian R, Heng-Moss T, Bradshaw J, Sarath G, Spilker C (2014) Evaluation of tetraploid switchgrass populations (*Panicum virgatum* L.) for host suitability and differential resistance to four cereal aphids. *J Econ Entomol* 107: 424-431.
16. Koch KG, Heng-Moss T, Bradshaw J, Sarath G (2014) Categories of resistance to greenbug and yellow sugarcane aphid (homoptera: aphididae) in three tetraploid switchgrass populations. *Bioenergy Research*. E-Pub ahead of print.
17. Kovacs FA, Sarath G, Woodworth K, Twigg P, Tobias CM (2013) Abolishing activity against ascorbate in a cytosolic ascorbate peroxidase from switchgrass. *Phytochemistry* 94: 45-52.
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28. Pedersen, J.F., Sattler, S.E., Anderson, W.F. 2013. Evaluation of public sweet sorghum A-lines for use in hybrid production. *BioEnergy Research*. 6: 91-102. (DOI:10.1007/s12155-012-9231-1) (ARIS log # 278035)
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28. Mitchell, R.B., S. Harlow. Switchgrass stand establishment: key factors for success, Fact Sheet No. 4, CenUSA Bioenergy, ISU, Ames, IA. (www.cenusa.iastate.edu). 2013 (Ext. Circular).

Dairy Forage and Aquaculture Research Unit (DFARU), Madison, WI (NP215)

Project Title: Improving bioenergy and forage plants and production systems for the central U.S.
Project No: 3655-21000-056-00D

Accomplishments

Secrets of switchgrass evolution revealed. The choice of switchgrass as a national herbaceous model species for bioenergy feedstock development has led to an increase in the number of USA breeding programs from two in 1995 to 12 in 2012. Collaborative research between ARS, The Samuel Roberts Noble Foundation, and the DOE Great Lakes Bioenergy Research Center characterized genetic diversity in switchgrass across its native range in the USA. They demonstrated the existence of at least 10 distinct types of switchgrass, ranging from highly drought-tolerance ecotypes of the Great Plains to highly heat-tolerant types of the Atlantic Seaboard. They showed that all unique types of switchgrass originate in particular regions in the southern USA where switchgrass survived numerous Ice Ages. These results have significant implications for using a wide range of genetic and geographic diversity in many breeding programs and point to the distinct possibility of combining important biomass production traits such as late flowering, early nutrient recycling, and winter hardiness.

Natural hybrids reveal pathway to increased biomass yield and adaptation of switchgrass. While cellulosic biomass crops are receiving considerable research attention, economic and life-cycle analyses uniformly indicate that low biomass yield is a major factor limiting adoption and deployment of new cultivars. Collaborative research between ARS, The Samuel Roberts Noble Foundation, and the DOE Great Lakes Bioenergy Research Center led to the discovery of the first documented natural hybrids between the two dominant ecotypes of switchgrass: upland and lowland ecotypes. Researchers showed that natural hybrids were created during the Ice Ages when upland and lowland types shared the same habitats. They demonstrated that these hybrids are stable, able to survive under a wide range of conditions, and capable of sexual reproduction and seed production. This research forms the basis for broadening breeding programs to utilize natural hybrids and/or to create new high-yielding and broadly adaptive hybrids between diverse ecotypes of switchgrass, increasing biomass yield and adaptation across a broad landscape.

DNA Sequencing platform reveals 1.4 million markers available for genomic selection.

Genomic selection is a mechanism to conduct selection and breeding of crops directly on the genes of interest, using information from the entire genome of a plant. ARS participated with the University of Wisconsin, Michigan State University, and the U.S. Department of Energy Great Lakes Bioenergy Research Center to develop a mechanism to routinely assay thousands of switchgrass genotypes for DNA markers that represent coding regions of 169,000 unique genes. This technology is currently being applied to germplasm that represents six switchgrass breeding in various regions of the USA. The DNA probe set and genomic selection prediction equations will be made available to numerous switchgrass breeding programs in a community-wide effort to improve the rate of gain for increasing biomass yield of switchgrass.

Late-flowering switchgrass boosts biomass production in the northern USA. Switchgrass populations that flower 4 to 5 weeks later than local local populations in the northern USA are capable of accumulating biomass through the growing season, up to the time of killing frost. It has been hypothesized that development of winter-hardy and late-flowering switchgrass

populations will be an effective mechanism to increase sustainable biomass productivity of this species. In collaborative efforts between Lincoln, NE and Madison, WI, two multi-location field experiments documented increases of up to 45% in dry biomass yield from two late flowering populations bred for increased biomass yield and winter survival. One of these has been released as the cultivar Liberty. The yield gains were documented at locations within USDA hardiness zones 3 through 5, verifying the ability of these populations to withstand severe winters, despite the late flowering trait.

Developing late-flowering big bluestem for biomass production in the northern USA. Big bluestem (*Andropogon gerardii*) has been largely ignored on the national scale, but has greater drought tolerance and potentially higher biomass yield than switchgrass. Three late-flowering big bluestem synthetic populations were developed from field evaluations of germplasm collected through the range of big bluestem. The populations flower 5 to 7 weeks later than local big bluestem in southern Wisconsin, allowing them to continue accumulating biomass through the growing season, up to the time of killing frost. These populations have been planted in field experiments to evaluate their potential for sustainable biomass production.

Optimizing management of reed canarygrass for biomass production. Reed canarygrass (*Phalaris arundinacea*) is a cool-season perennial grass with potential as a biomass energy crop. It can be grown in a wide range of soils and environments and has been used as a pasture and hay crop for many decades. The number and timing of harvests during a growing season directly affect biomass yield and biofuel quality. Research demonstrated that biomass yield was highest for a 2-cut harvest management with first cut made on or near the summer solstice and the last cut made after a killing frost. Biomass that was allowed to stand over winter had much superior quality, especially for combustion applications, but biomass yield was reduced by up to 60% on average and was sometimes impossible to harvest due to snow packing. This experiment provides valuable information on average reed canarygrass biomass yields and quality for several harvest management systems.

Discovery and evaluation of reed canarygrass germplasm for biomass production. Reed canarygrass has been used as a forage crop for many years. Because of its high productivity and its persistence, it is being considered for development as a dedicated biomass feedstock crop. This will require many years of intensive selection and breeding to produce new varieties with the required traits. Thus, it is important to begin with the best plant materials, some of which were identified in this study. Wild populations had the highest biomass yield compared to varieties bred for forage production systems, indicating that there is considerable potential to increase biomass yields of this species by selection and breeding.

Funded Bioenergy Grants: Grants linked to the parent CRIS Project 3655-21000-056-00D.

1. 2008-2010. "US native grass breeding consortium to identify regional optimum biomass productivity on marginal lands," U.S. Department of Energy. PI: Stacy Bonos, Rutgers University, *Award* to Michael Casler (Co-PI): \$163,000.
2. 2008-2010. "Small-scale pelletization of switchgrass for bioenergy," U.S. Department of Energy and Bay Mills Indian Community. *Award* to Michael Casler (PI): \$10,000.
3. 2008-2013. "Develop improved big bluestem and lowland-type switchgrass germplasm for the northern USA," CRADA with Forage Genetics, Inc. *Award* to Michael Casler (PI): \$300,000.

4. 2009-2017. “Translational research and breeding in switchgrass using maize as a model discovery engine,” U.S. Department of Energy, Great Lakes Bioenergy Research Center. PI: Timothy Donohue, University of Wisconsin, *Award* to research team on which Michael Casler is a non-funded cooperator: \$2.4M.
5. 2011-2016. “Sustainable production and distribution of bioenergy for the Central USA,” U.S. CAP grant from USDA-NIFA. *Award* to Michael Casler (CoPI): \$1.5M.
6. 2013-2016. “Genetic control of flowering in switchgrass,” U.S. Department of Energy. *Award* to Michael Casler (PI): \$377,000.

Selected Publications (2010-2014)

1. Casler, M.D. and K.P. Vogel. 2014. Selection for biomass yield in upland, lowland, and hybrid switchgrass. *Crop Sci.* 54:626-636.
2. Price, D.L. and M.D. Casler. 2014. Predictive relationships between plant morphological traits and biomass yield of switchgrass. *Crop Sci.* 54:637-645.
3. Price, D.L. and M.D. Casler. 2014. Inheritance of secondary morphological traits for among- and within-family selection in upland tetraploid switchgrass. *Crop Sci.* 54:646-653.
4. Price, D.L. and M.D. Casler. 2014. Divergent selection for secondary traits in upland tetraploid switchgrass and effects on sward biomass yield. *BioEnergy Res.* 7:329-337.
5. Nelson, M.F., N.O. Anderson, M.D. Casler, and A.R. Jakubowski. 2014. Population genetic structure of N. American and European *Phalaris arundinacea* L. as inferred from inter-simple sequence repeat markers. *Biol. Invasions* 16:353-363.
6. Jakubowski, A.R., R.D. Jackson, and M.D. Casler. 2014. The history of reed canarygrass in North America: Persistence of natives among invading Eurasian populations. *Crop Sci.* 54:210-219.
7. Resende, R.M.S., M.D. Casler, and M.V. de Resende. 2014. Genomic selection in forage breeding: Accuracy and methods. *Crop Sci.* 54:143-156.
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9. Vogel, K.P., R.B. Mitchell, G. Sarath, H.G. Jung, B.S. Dien, and M.D. Casler. 2013. Switchgrass biomass composition altered by six generations of divergent breeding for digestibility. *Crop Sci.* 53:853-862.
10. Casler, M.D., and A.J. Smart. 2013. Plant mortality and natural selection may increase biomass yield in switchgrass swards. *Crop Sci.* 53:500-506.
11. Olmstead, J., M.D. Casler, and E.C. Brummer. 2013. Genetic variability for biofuel traits in a circumglobal reed canarygrass collection. *Crop Sci.* 53:524-531.
12. Lu, F., A.E. Lipka, J. Glaubitz, R. Elshire, J.H. Cherney, M.D. Casler, E.S. Buckler, and D.E. Costich. 2013. Switchgrass genomic diversity, ploidy, and evolution: Novel insights from a network-based SNP discovery protocol. *PLoS Genetics* 9(1):e1003215.
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13. Jakubowski, A.R., M.D. Casler, and R.D. Jackson. 2013. Genetic evidence suggests a widespread distribution of native North American populations of reed canarygrass. *Biol. Invasions* 15:261-268.
14. Price, D.L. and M.D. Casler. 2012. Simple regression models as a threshold for selecting AFLP loci with reduced error rates. *BMC Bioinformatics* 13:268. *doi:10.1186/1471-2105-13-268*

15. Ersoz, E.S., M.H. Wright, J.L. Pangilinan, M.J. Sheehan, C. Tobias, M.D. Casler, E.S. Buckler, and D.E. Costich. 2012. SNP Discovery with EST and NextGen sequencing in switchgrass (*Panicum virgatum* L.). PLOS One 7(9):e4412. doi:10.1371/journal.pone.0044112
16. Dien, B.S., M.D. Casler, R.E. Hector, L.B. Iten, N.N. Nichols, J.A. Mertens, and M.A. Cotta. 2012. Biochemical processing of reed canarygrass into fuel ethanol. Intl. J. Low-Carbon Tech. doi:10.1093/ijlct/ctr041
17. Schmer, M.R., K.P. Vogel, R.B. Mitchell, B.S. Dien, H.G. Jung, and M.D. Casler. 2012. Temporal and spatial variation in switchgrass biomass composition and theoretical ethanol yield. Agron. J. 104:54-64.
18. Price, D.L., P.R. Salon, and M.D. Casler. 2012. Big bluestem gene pools in the Central and Northeastern United States. Crop Sci. 52:189-200.
19. Jakubowski, A.R., R.D. Jackson, R.C. Johnson, J. Hu, and M.D. Casler. 2011. Genetic diversity and population structure of Eurasian populations of reed canarygrass: cytotypes, cultivars, and interspecific hybrids. Crop Pasture Sci. 62:982-991.
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21. Jakubowski, A.R., M.D. Casler, and R.D. Jackson. 2011. Has selection for improved agronomic traits made reed canarygrass invasive? PLoS One 6:e25757.
22. Zhang, Y., J. Zalapa, A.R. Jakubowski, D.L. Price, A. Acharya, Y. Wei, E.C. Brummer, S.M. Kaeppler, and M.D. Casler. 2011. Post-glacial evolution of *Panicum virgatum*: Centers of diversity and gene pools revealed by SSR markers and cpDNA sequences. Genetica 139:933-948.
23. Zhang, Y., J. Zalapa, A.R. Jakubowski, D.L. Price, A. Acharya, Y. Wei, E.C. Brummer, S.M. Kaeppler, and M.D. Casler. 2011. Natural hybrids and gene flow between upland and lowland switchgrass. Crop Sci. 51:2626-2641.
24. Tahir, M.H.N., M.D. Casler, K.J. Moore, and E.C. Brummer. 2011. Biomass yield and quality of reed canarygrass under five harvest management systems for bioenergy production. Bioenergy Res. 4:111-119.
25. Vogel, K.P., B.S. Dien, H.G. Jung, M.D. Casler, S.D. Masterson, and R.B. Mitchell. 2011. Quantifying actual and theoretical ethanol yields for switchgrass strains using NIRS analysis. Bioenergy Res. 4:96-110.
26. Zalapa, J.E., D.L. Price, S.M. Kaeppler, C.M. Tobias, M. Okada, and M.D. Casler. 2011. Hierarchical classification of switchgrass genotypes using SSR and chloroplast sequences: ecotypes, ploidy, gene pools, and cultivars. Theor. Appl. Genet. 122:805-817.
27. Casler, M.D. 2010. Genetics, breeding, and ecology of reed canarygrass. Intl. J. Plant Breed. 4:30-36.
28. Costich, D.E., B. Friebe, M.J. Sheehan, M.D. Casler, and E.S. Buckler. 2010. Genome-size variation in switchgrass (*Panicum virgatum*): Flow cytometry and cytology reveal rampant aneuploidy. Plant Genome 3:130-141.
29. Jakubowski, A.R., M.D. Casler, and R.D. Jackson. 2010. The benefits of harvesting wetland invaders for cellulosic biofuel: An ecosystem services perspective. Restoration Ecol. 18:789-795.
30. Jakubowski, A.R., M.D. Casler, and R.D. Jackson. 2010. Landscape context predicts reed canarygrass invasion: Implication for management. Wetlands 30:685-692.

31. Shinnars, K.J., G.C. Boettcher, R.E. Muck, P.J. Weimer, and M.D. Casler. 2010. Harvest and storage of two perennial grasses as biomass feedstocks. *Trans. Amer. Soc. Agric. Biol. Eng.* 53:359-370.
32. Digman, M.F., K.J. Shinnars, M.D. Casler, B.S. Dien, R.D. Hatfield, H.J. Jung, R.E. Muck, and P.J. Weimer. 2010. Optimizing on-farm pretreatment of perennial grasses for fuel ethanol production. *Bioresource Tech.* 101:5305-5314.
33. Casler, M.D. 2010. Changes in mean and genetic variance during two cycles of within-family selection in switchgrass. *BioEnergy Res.* 3:47-54.

Other Publications

1. E.C. Brummer and M.D. Casler. 2014. Cool-season forages. (*in press*) *In* S. Smith et al. (eds.) Genetic gain in major U.S. field crops. CSSA Spec. Publ. 33. ASA-CSSA-SSSA, Madison, WI.
2. Mitchell, R.B., D.K. Lee, and M.D. Casler. 2014. Switchgrass. p. 75-89 *In* D.L. Karlen (ed) Cellulosic energy cropping systems. John Wiley & Sons, Ltd., Chichester, UK.
3. Jakubowski, A.R. and M.D. Casler. 2014. Regional gene pools for restoration, conservation, and genetic improvement of prairie grasses. p. 67-80 *In* M.C. McCann et al. (eds.) Plants and Bioenergy. *Advances in Plant Biology* 4, Springer, New York.
4. Casler, M.D. 2013. Creating dedicated bioenergy crops. p. 77-82 *In* S. Bittman et al. (eds.) Advanced forage management. Farmwest.com, Abbotsford, BC, Canada.
5. Casler, M.D., R.B. Mitchell, and K.P. Vogel. 2012. Switchgrass. p. 563-590 *In* C. Kole et al. (eds.) Handbook of bioenergy crop plants Vol. 2. Taylor & Francis, New York.
6. Casler, M.D. 2012. Switchgrass breeding, genetics, and genomics. p. 29-54 *In* A. Monti (ed.) Switchgrass. Springer, New York.
7. Parrish, D.J., M.D. Casler, and A. Monti. 2012. The evolution of switchgrass as an energy crop. p. 1-28 *In* A. Monti (ed.) Switchgrass. Springer, New York.

Bioenergy Research Unit, Peoria, IL

Project Title: Advanced conversion technologies for sugars and biofuels: superior feedstocks, pretreatments, inhibitor removal, and enzymes

Project No: 3620-41000-133-00D

Accomplishments

- 1. Evaluated switchgrass fractions to determine how ethanol yield varies by anatomical fraction.** It was our hypothesis that targeting specific anatomical parts of switchgrass plants will allow for more directed breeding for biomass conversion quality. Switchgrass samples were segregated into internodes and leaves. Samples were analyzed for carbohydrates and lignin contents and processed using dilute-acid followed by either enzymatic release of sugars or simultaneous saccharification and fermentation to ethanol using commercial cellulases and *Saccharomyces* yeast. Results for the sheath and internodes were highly correlated with Klason lignin contents, however, the leaves gave higher than expected ethanol yields. The hypothesis was confirmed using a set of switchgrass grass cultivars bred for increased forage quality; higher conversion quality using our fermentation assay was detected for stems only but not whole plants. This last result is included for a manuscript in preparation and the earlier result has been presented at meetings.
- 2.**
- 3. Evaluated five warm season perennial forage grasses for sugar yields.** This was a project led by Dr. John Read (USDA-ARS, Crop Science Research Laboratory, MS State, MS). Dr. Read earlier conducted a field study that compared 5 grass hay crops for manure-nutrient management in a swine effluent spray field. All five perennials were evaluated at various cuttings to select the most promising for ethanol production. This study included the development of a novel conversion assay suitable for small sample sizes. Results are being presented at a USDA co-sponsor workshop.
- 4. Evaluated alfalfa stems for conversion to ethanol .** Alfalfa has the potential to serve a bioenergy crop in rotation with corn. It adds nitrogen and organic carbon back to the soil, enhances the next corn yields by 5-10%, and the leaves are easily fractionated and marketed for animal feed. Information on conversion of alfalfa stems is lacking from the literature. Alfalfa was converted at the laboratory scale with an ethanol yield of 204-241 liters per ton using a commercially feasible ammonium pretreatment and a NCAUR developed yeast strain. Furthermore, new alfalfa lines, commercial development, with altered lignin composition were evaluated for their ability to enhance conversion yields. The results showed that the COMT genotype improved both sugar and ethanol yields. Finally, 112 alfalfa samples were screened for ethanol yields; ethanol yields were poorly correlated with lignin contents, indicating that other targets might be needed from improving alfalfa stem conversion quality. This work was the subject of two invited presentations – sponsored by the National Alfalfa and Forage Association and Consortium for Alfalfa Improvement, one invited departmental seminar at Toledo University (Department of Chemical Engineering, Toledo, OH), a poster presentation, and one submitted publication.
- 5. Select pretreatment methodology for converting reed canary grass into ethanol.** Reed canary grass (RCG) is grown for forage in Northern United States and is being investigated as a bioenergy crop by ARS researchers (Madison, WI). Three different pretreatments were evaluated for RCG conversion and dilute-ammonium was selected as the most promising. Pretreatment conditions were optimized, evaluated on biomass collected at two different harvest

maturities, and converted to ethanol using a xylose-fermenting yeast strain. The final ethanol yield was the highest conversion efficiency ever reported for this biomass. The work is the subject of an invited peer-reviewed paper to be published in a special journal issue published by Oxford.

6. **Determined the effects of ear rot damaged kernels upon corn conversion to ethanol and distillers grains.** Stenocarpella is a leading cause of corn ear rot within the Midwest and is increasing in prevalence. Despite the rapid growth in corn ethanol production, nothing is known regarding the effect of processing infected kernels into ethanol. This work led to some important conclusions. Corn ethanol yield was unaffected. However, the animal feed co-produced with the corn ethanol (distillers' dried grains with solubles) was altered; most significantly it was lowered in oil content. Stenocarpella infected corn is not considered a risk for feeding to production animals. Therefore, Stenocarpella infected corn will not alter ethanol yields but may lower DDGS oil contents and, therefore, its energy content. The work was the subject of two invited oral presentations, invited proceedings, and a submitted manuscript.
7. **Analyze switchgrass samples for fermentation yields to ethanol with the goal of selecting superior cultivars for conversion.** We have analyzed 100+ switchgrass samples for conversion to ethanol. This data has been used to develop a method for measuring ethanol yield using near-infrared spectroscopy (NIR) by our collaborators. This method is directly being used by our ARS plant breeders to improve switchgrass conversion. The method has also been adopted by an official NIR user group and distributed to multiple laboratories. We have also worked to screen switchgrass samples from a traditional switchgrass breeding program demonstrating that this method is effective for improving biomass quality. This work led the important conclusion that analyzing fractionated stems led to different results than using whole biomass.
8. **Develop an integrated process for converting switchgrass into ethanol.** Dilute-ammonium hydroxide has been successfully developed as a pretreatment process for switchgrass. Pretreated switchgrass samples were evaluated for conversion to sugars following enzymatic digestion and to ethanol using a combination of enzymes and an in-house xylose-fermenting *S. cerevisiae*. Glucose and ethanol yields were up to 94.2 and 82.9% of maximum ethanol. Currently an improved pretreatment process is being developed that reduces water usage by 400% and reaction temperature from 180°C to 110°C. It is envisioned that this pretreatment will be suitable for distributed processing.
9. **Begin to analyze lipid producing yeast for the next project plan.** A convenient and rapid chemical spectrophotometer method has been adapted for measuring lipid contents in yeast. This method is being shared with Dr. Slininger. Yeasts from the culture collection have also been screened for their ability to produce lipids from glucose and two selected for further study on biomass hydrolysates. This work is directed towards the next project plan to add an element for "drop in fuels". A method to selectively isolate yeast from corn steep liquor failed but resulted in yeast isolates that will be shared with Dr. Hector. One of identified yeasts from the culture collection was submitted and accepted by the patent committee as a patent proposal (PI Dr. Slininger). We also presented the data as a poster at a technical meeting.
10. **Analyze Napier grass fermentation data.** Approximately 100 Napier plant samples have been ranked for ethanol yield using our single-bottle dilute acid pretreatment and simultaneous saccharification and fermentation assay. The protocol was modified to extract soluble sugars prior to pretreatment and to analyze the leaves and stems separately. The results demonstrated that stems were over 50% more resistant to enzymatic conversion of cellulose compared to the leaves. This suggests that a more accurate screen would focus on stem material.

11. **Began developing (expected) low capital cost moist ammonium pretreatment for switchgrass and Napier grass.** In particular, field grown Napier grass samples were pretreated with ammonia at 20% at 110°C for 2 days and 40% moisture. Glucose and xylose enzymatic conversion efficiencies were 59– 93% glucose and 53-61% monomeric xylose. We envision that this technology can be introduced at the distributed farm level and perhaps compressed for convenient shipment to centralized refineries.
12. **Improving pennycress for bioenergy.** *Thlaspi arvense* (pennycress) germplasm was improved from 2% non-dormancy to 87% non-dormancy through 3 generations of selection pressure in a growth chamber. Seed for this selection is now being seed increased for a field plot evaluations. One of the main challenges with the domestication of pennycress is the post-harvest maturation requirement (dormancy). This dormancy has an initial period of 3 months and full non-dormancy of seed requires 12 months of storage. Because of this dormancy, pennycress can place seed within the soil profile for many years rather than immediately germinate and die under the summer canopy which would out-compete it. Secondly, non-dormant seed will allow commercial planting of seed in the first growing season post-harvest rather than a 15 month storage cycle which is now required before planting.

Funded Bioenergy Grants

1. Breeding and selection of napiergrass (*Pennisetum purpureum*) for conversion to biofuels. 2010-2015 with BP Biofuels North America, LLC (\$48,125).
2. Fermentation of dry fractionated grits (with different germ amount) for dry grind ethanol production. 2012-2014 with Gevo, Inc. and cooperate research agreement with Dr. Vijay Singh, University of Illinois (\$12,000).
3. Sustainable production and distribution of bioenergy for the Central USA. 2011-2014 with Iowa State, NIFA Coordinated Agricultural Project (CAP) Grant, supported by Agriculture and Food Research Initiative, USDA National Institute of Food and Agriculture (\$25,000,000 total budget) (\$500,000).
4. Distribution and maintenance of calibration for chemical composition and conversion quality of switchgrass. Near Infrared Spectroscopy Consortium (NIRSC). (Non-funded CRADA).
5. Accelerated Commercial Development of Hydrotreated Renewable Jet Fuel (HRJ) from Redesigned Oilseed Feedstocks Supply Chains, NIFA, 2012-2016, PI: T. Isbell \$6,998,790.

Selected Publications (2010 – 2014)

1. Anderson, W. F., Dien, B. S., Jung, H-J. G., Vogel, K. P., and Weimer, P. J. Effects of forage quality and cell wall constituents of Bermuda grass on biochemical conversion to ethanol. *Bioenerg. Res.* 3:225-237. 2010.
2. Arora, A., Dien, B. S., Belyea, R. L., Singh, V., Tumbleson, M. E., and Rausch, K. D. Nutrient recovery from the dry grind process using sequential micro and ultrafiltration of thin stillage. *Bioresource Technol.* 101:3859-3863. 2010.
3. Arora, A., Dien, B. S., Belyea, R. L., Singh, V., Tumbleson, M. E., and Rausch, K. D. Heat transfer fouling characteristics of microfiltered thin stillage from the dry grind process. *Bioresource Technol.* 101:6521-6527. 2010.
4. Digman, M. F., Shinnars, K. J., Casler, M. D., Dien, B. S., Hatfield, R. D., Jung, H-J. G., Muck, R. E., and Weimer, P. J. Optimizing on-farm pretreatment of perennial grasses for fuel ethanol production. *Bioresource Technol.* 101:5305-5314. 2010.

5. Kim, Y., Hendrickson, R., Mosier, N. S., Ladisch, M. R., Bals, B., Balan, V., Dale, B. E., Dien, B. S., and Cotta, M. A. Effect of compositional variability of Distillers' Grains on cellulosic ethanol production. *Bioresource Technol.* 101:5385-5393. 2010.
6. Nichols, N. N., Dien, B. S., and Cotta, M. A. Fermentation of bioenergy crops into ethanol using biological abatement for removal of inhibitors. *Bioresource Technol.* 101(19):7545-7550. 2010.
7. Qureshi, N., Saha, B. C., Hector, R. E., Dien, B. S., Hughes, S. R., Liu, S., Iten, L. B., Bowman, M. J., Sarath, G., and Cotta, M. A. Production of butanol (a biofuel) from agricultural residues: Part II—Use of corn stover and switchgrass hydrolysates. *Biomass Bioenergy* 34:566-571. 2010.
8. Ximenes, E. A., Kim, Y., Mosier, N., Dien, B. S., and Ladisch, M. R. Inhibition of cellulases by phenols. *Enzyme Microbiol. Technol.* 46:170-176. 2010.
9. Zhu, J. Y., Zhu, W., O'Bryan, P. J., Dien, B. S., Tian, S., Gleisner, R., and Pan, X. J. Ethanol production from SPORL-pretreated lodgepole pine: preliminary evaluation of mass balance and process energy efficiency. *Appl. Microbiol. Biotechnol.* 86:1355-1365. 2010.
10. Arora, A., Seth, A., Dien, B. S., Belyea, R. L., Singh, V., Tumbleson, M. E., and Rausch, K. D. Microfiltration of thin stillage: process simulation and economic analyses. *Biomass Bioenergy* 35:113-120. 2011.
11. Bowman, M. J., Dien, B. S., O'Bryan, P. J., Sarath, G., and Cotta, M. A. Selective chemical oxidation and depolymerization of switchgrass (*Panicum virgatum* L.) xylan with oligosaccharide product analysis by mass spectrometry. *Rapid Commun. Mass Spectrom.* 25:941-950. 2011.
12. Dien, B. S., Miller, D. J., Hector, R. E., Dixon, R. A., Chen, F., McCaslin, M., Risen, P., Sarath, G., and Cotta, M. A. Enhancing alfalfa conversion efficiencies for sugar recovery and ethanol production by altering lignin composition. *Bioresource Technol.* 102:6479-6486. 2011.
13. Easson, M. W., Condon, B. D., Dien, B. S., Iten, L. B., Slopek, R. P., Yoshioka-Tarver, M., Lambert, A. H., and Smith, J. N. The application of ultrasound in the enzymatic hydrolysis of switchgrass. *Appl. Biochem. Biotechnol.* 16:1322-1331. 2011.
14. Hector, R. E., Dien, B. S., Cotta, M. A., and Qureshi, N. Engineering industrial *Saccharomyces cerevisiae* strains for xylose fermentation and comparison for switchgrass conversion. *J. Ind. Microbiol. Biotechnol.* 38(9):1193-1202. 2011.
15. Nichols, N. N., Sutivisedsak, N., Dien, B. S., Biswas, A., Lesch, W. C., and Cotta, M. A. Conversion of starch from dry common beans (*Phaseolus vulgaris* L.) to ethanol. *Ind. Crops Prod.* 33:644-647. 2011.
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18. Vogel, K. P., Dien, B. S., Jung, H. G., Casler, M. D., Masterson, S. D., and Mitchell, R. B. Quantifying actual and theoretical ethanol yields for switchgrass strains using NIRS analyses. *Bioenerg. Res.* 4:96-110. 2011.
19. Ximenes, E. A., Kim, Y., Mosier, N., Dien, B. S., and Ladisch, M. R. Deactivation of cellulases by phenols. *Enzyme Microbiol. Technol.* 48:54-60. 2011.

20. Agler, M. T., Werner, J. J., Iten, L. B., Dekker, A., Cotta, M. A., Dien, B. S., and Angenent, L. T. Shaping reactor microbiomes to produce the fuel precursor n-butyrate from pretreated cellulosic hydrolysates. *Environ. Sci. Technol.* 46:10229-10238. 2012.
21. Bowman, M. J., Dien, B. S., Hector, R. E., Sarath, G., and Cotta, M. A. Liquid chromatography-mass spectrometry investigation of enzyme-resistant xylooligosaccharides structures of switchgrass associated with ammonia pretreatment, enzymatic saccharification, and fermentation. *Bioresource Technol.* 110:437-447. 2012.
22. Bowman, M. J., Dien, B. S., O'Bryan, P. J., Sarath, G., and Cotta, M. A. Comparative analysis of end point enzymatic digests of arabino-xylan isolated from switchgrass (*Panicum virgatum* L) of varying maturities using LC-MS⁽ⁿ⁾. *Metabolites* 2:959-982. 2012.
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24. Dien, B. S., Casler, M. D., Hector, R. E., Iten, L. B., Nichols, N. N., Mertens, J. A., and Cotta, M. A. Biochemical processing of reed canarygrass into fuel ethanol. *Int. J. Low-Carbon Technol.* 7:338-347. 2012.
25. Dien, B. S., Wicklow, D. T., Singh, V., Moreau, R. A., Winkler-Moser, J. K., and Cotta, M. A. Influence of *Stenocarpella maydis* infected corn on the composition of corn kernel and its conversion into ethanol. *Cereal Chem.* 89(1):15-23. 2012.
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27. Qureshi, N., Dien, B. S., Liu, S., Saha, B. C., Cotta, M. A., Hughes, S. R., and Hector, R. E. Genetically engineered *Escherichia coli* FBR5: Part II. Ethanol production from xylose and simultaneous product recovery. *Biotechnol. Prog.* 28:1179-1185. 2012.
28. Qureshi, N., Dien, B. S., Liu, S., Saha, B. C., Hector, R. E., Cotta, M. A., and Hughes, S. R. Genetically engineered *Escherichia coli* FBR5: Part I. Comparison of high cell density bioreactors for enhanced ethanol production from xylose. *Biotechnol. Prog.* 28:1167-1178. 2012.
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32. Chen, M.-H., Kaur, P., Dien, B. S., Below, F., Vincent, M. L., and Singh, V. Use of tropical maize for bioethanol production. *World J. Microbiol. Biotechnol.* 29:1509-1515. 2013.
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44. Slininger, P. J., Dien, B. S., Lomont, J. M., Bothast, R. J., Ladisch, M. R., and Okos, M. R. Evaluation of a kinetic model for computer simulation of growth and fermentation by *Scheffersomyces (Pichia) stipitis* fed D-xylose. *Biotech. Bioeng.* (accepted: March 2014).

Pasture Systems and Watershed Management Research Unit, University Park, PA
Project Title: Multifunctional Farms and Landscapes to Enhance Ecosystem Services
Project Number: 1902-21000-008-00D

Accomplishments

Potential for biofuel production from Northeastern CRP lands. Concerns about finding sufficient land for biofuel production has experts eyeing marginal croplands that have been placed in the Conservation Reserve Program (CRP). An extensive study by ARS scientists of grassland sites across major Northeastern ecoregions determined the effects plant species composition, diversity, above-ground biomass, and chemical composition had on potential biofuel yield. This study showed that CRP lands with a high proportion of native warm-season prairie grasses have the potential to produce more than 600 gallons of ethanol per acre while still maintaining the ecological benefits of grasslands.

Switchgrass still productive after 20 years of management. Switchgrass, a native warm-season perennial grass, has received extraordinary attention as a candidate cellulosic bioenergy crop. Despite being native, there is little information on its long-term (>10 years) persistence and performance. USDA-ARS scientists in University Park, PA measured the biomass yields and plant density of experimental switchgrass germplasm and the standard cultivar Cave-in-Rock after 20 years of management. Biomass yields of all switchgrasses were stable and stand density was relative high after 20 years demonstrating the long-term sustainability of switchgrass as a bioenergy crop. These are the first long-term data on the experimental germplasm (since released as the cultivars BoMaster and Peformer by ARS in Raleigh, NC) and indicate that southerly adapted lowland cultivars can provide diversity in cultivar choices for switchgrass bioenergy production in the northeastern U.S. (Sanderson, 2010). BoMaster and Performer are now marketed by Ernst Conservation Seeds in Meadville, Pennsylvania.

Carbon sequestration potential of a switchgrass bioenergy crop. Switchgrass is an important bioenergy crop with the potential to provide a reliable supply of renewable energy while also removing carbon dioxide from the atmosphere and sequestering it in the soil. ARS scientists at University Park monitored biomass production and carbon dioxide fluxes during the first four years following switchgrass establishment. Averaged over the first four years of production, this switchgrass field was a net sink of 142 g CO₂ m⁻² yr⁻¹ (39 g C m⁻² yr⁻¹). Photosynthetic C uptake, ecosystem respiration and evapotranspiration were all lower than results commonly observed in the Midwest, primarily due to lower growing-season temperature and lower available solar radiation (Skinner and Adler, 2010). In addition to their primary function as a source of renewable energy, switchgrass bioenergy crops in the northeastern USA can sequester carbon dioxide during, at least, the first few years following establishment.

Switchgrass establishment date and weed control method affect yield. Controlling weeds is important for accelerating biomass production from switchgrass, however, since it is a new bioenergy crop, few chemical weed management options are available. We tested both approved and new chemicals and establishment time as methods to control weeds. Agricultural Research Service (ARS) and Penn State University scientists found that when a combination of new and approved chemicals were used to control weeds, the earlier seeding date yielded more biomass. However, with a later seeding date, when weed pressure was lower, all treatment methods were

equally effective. This study highlights the importance of identifying new weed management strategies to maximize the yields of switchgrass.

GHG mitigation strategies for bioenergy feedstock production. State and federal regulations reward innovation for improvements in the life cycle greenhouse gas (GHG) emissions of the fuel pathway and the type of feedstock chosen for conversion to biofuel. However, there is not an incentive strategy in place to reward the further reduction of GHG emissions from production of a particular feedstock. Agricultural Research Service (ARS), National Renewable Energy Laboratory, Drexel University, and DuPont scientists reviewed and analyzed data from GHG life cycle assessments, demonstrating that feedstock production can contribute more than 50% of the total GHG emissions. Instead of tracking all the components of life cycle GHG emissions in feedstock production, which would be overwhelming, we identified the most important components contributing to GHG emissions which have potential for mitigation, N fertilizer material, N₂O emissions, and tillage impact on soil carbon. This study provides a practical path forward to capture further reductions in life cycle GHG emissions by adopting the identified mitigation strategies.

Savings achieved when displacing fuel oil with densified switchgrass. Many studies focus on quantifying the life cycle greenhouse gas (GHG) emissions of biofuel use without considering the economic implications. Given that biomass is a limited resource, we consider both in evaluating its displacement of fuel oil, natural gas, and coal. Agricultural Research Service (ARS), Drexel University, and Penn State University scientists found that switchgrass 1) was a cheaper fuel than fuel oil (could save consumers in NE US \$2.3 – \$3.9 billion annually), 2) displaces more than twice as much petroleum when replacing fuel oil compared with gasoline, and 3) is a cheaper GHG mitigation strategy when it replaces fuel oil rather than electricity in the NE US (reduces GHGs at a cost savings of \$10 – 11.6 billion annually). This study highlights the importance of explicitly targeting GHG reductions and petroleum offsets so biomass is not distributed towards more expensive options, such as the electricity sectors as with RPS legislations.

Reducing the carbon footprint of cellulosic ethanol. After producing ethanol from crop residues such as corn stover and straw, a slowly decomposing byproduct remains which is typically burned for energy recovery, but harvesting crop residues can result in decreased crop yields and soil carbon levels. Agricultural Research Service (ARS) and Drexel University scientists compared the current practice of burning this residue, to applying it back to the land. They found that although most studies have recommended burning this material to generate electricity for the biorefinery, applying it to the land instead resulted in ethanol with the lowest greenhouse gas footprint and could be cheaper for farmers and the biorefinery. This finding could help the industry evaluate the different markets for byproducts produced at the biorefinery, considering both the economic and environmental impacts.

Funded Bioenergy Grants

1. 2008-2013. USDA-NIFA, Biomass Research and Development Initiative. US Native Grass Breeding Consortium to Identify Regional Optimum Biomass Productivity on Marginal Land. (Co-PI) \$971,799.

2. 2008-2013. USDOE Sun Grant Initiative. Regional Biomass Feedstock Partnership – Corn Stover Residue Removal. (Co-PI). \$230,000.
3. 2008-2010. US Department of Transportation, Northeast Sun Grant Initiative. Breeding Perennial Grasses for Increased Biomass Production on Marginal Land. (Co-PI). \$79,261.
4. 2010-2012. Osage Bio Energy, LLC. Spatial analysis of soil carbon, N₂O emissions, and NO₃ leaching in a corn, winter barley, soybean cropping system. (PI). \$85,000.
5. 2010-2013. USDOE Sun Grant Initiative. Role of Conservation Grasslands as Bioenergy Feedstock. (PI). \$12,000.
6. 2011-2013. USDA-ARS Post doc “Growing bioenergy crops on marginal lands in the Northeast: Tradeoffs between greenhouse gas emission, carbon sequestration, and nitrate losses. (Co-PI). \$100,000.
7. 2011-2014. US Department of Transportation, Northeast Sun Grant Initiative. Production and life-cycle assessment of switchgrass across the heterogeneous landscape of the Northeast. (Co-PI) \$147,184.
8. 2011-2015. USDA-NIFA. Greenhouse gas life cycle analysis of biochar effects on marginal land conversion to switchgrass. (Co-PI). \$963,539.
9. 2011-2015. USDA-NIFA. Decision support tool for integrated biofuel greenhouse gas emission footprints. (Collaborator). \$799,000.
10. 2012-2016. USDA-NIFA, Biomass Research and Development Initiative. Lignocellulosic Biomass Conversion to Infrastructure Compatible Fuels, Products and Power. (Co-PI) \$7,000,000.

Selected Publications (2010-2014)

1. Skinner, R.H., and P.R. Adler. 2010. Carbon dioxide and water fluxes from switchgrass managed for bioenergy production. *Agric. Ecosys. Environ.* 138:257-264.
2. Sanderson, M.A. 2010. Long-term persistence of synthetic populations of a lowland switchgrass ecotype and the cultivar Cave-in-Rock. Online. *Forage and Grazing Lands*. doi:10.1094/FG-2010-0426-02-RS. www.plantmanagementnetwork.org.
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4. Curran, W.S., M.R. Ryan, M.W. Myers, and P.R. Adler. 2011. Effectiveness of sulfosulfuron and quinclorac for weed control during switchgrass establishment. *Weed Technology* 25:598-603.
5. Curran, W.S., M.R. Ryan, M.W. Myers, and P.R. Adler. 2012. Effects of seeding date and weed control on switchgrass establishment. *Weed Technology* 26:248-255.
6. Davis, S.C., W.J. Parton, S.J. Del Grosso, C. Keough, E. Marx, P.R. Adler, and E.H. DeLucia. 2012. Impact of second-generation biofuel agriculture on greenhouse-gas emissions in the corn-growing regions of the US. *Frontiers in Ecology and the Environment* 10(2): 69–74. <http://dx.doi.org/10.1890/110003>
7. Wilson, T. O., F. M. McNeal, S. Spatari, D. G. Abler, and P.R. Adler. 2012. Densified biomass can cost-effectively mitigate greenhouse gas emissions and address energy security in thermal applications. *Environmental Science and Technology* 46 (2):1270–1277.
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Northern Great Plains Research Laboratory (NGPRL), Mandan, ND

Project Titles: Rangeland and Livestock Resource Management; Integrated Agricultural Systems for the Northern Great Plains; Management Strategies to Sustainably Intensify Northern Great Plains Agroecosystems

Project Numbers: 5445-21310-001-00D, 5445-21660-002-00D, 5445-21660-003-00D

Accomplishments:

Crop residue supply. Crop residues, materials remaining in the field after grain harvest, are a promising abundant source of biomass for bioenergy production. However, it is important that harvesting crop residues does not harm the environment and is economically feasible for both farmers and biorefiners. ARS researchers at Mandan, North Dakota and Morris, Minnesota developed a method to determine the prices and amounts of biomass that could be profitably supplied to a local biorefinery. This technique identifies specific fields where biomass prices will be profitable. Results for a Minnesota biorefinery showed that farmers could begin to profitably deliver corn stover at prices above \$53 per ton, and that transportation costs result in crop residue harvest being concentrated near the biorefinery, concentrating environmental impacts near the facility, as well. These results provide farmers and biomass industry with information and an analytical method needed to evaluate the economic viability of using crop residues for energy production while avoiding negative environmental impacts.

Crop residue harvest economics. The biomass industry and farmers need to know how much biomass can be profitably produced, and this includes understanding how harvesting crop residue affects future crop production. The costs of producing crop residues can vary between sites with different soils, weather, and crop production systems. Data from field research studies at multiple locations are available in the publicly accessible REAPnet web site. A tool was built to retrieve data from the web site to generate production cost information from field studies in Iowa and North Dakota and compare the profitability of crop residue harvest strategies. Results show that biomass can be harvested at low removal rates with little short-term impact on crop productivity. Results also show that biomass can be harvested at lower costs at the lower harvest rates. However, it will be important to monitor longer-term changes to see if grain profitability decreases. Results showed that biomass could be profitably produced in the short-term at prices in the field of \$24-38 per dry ton at the Iowa site, and \$13-49 per dry ton at the North Dakota site. These results provide farmers and the biomass industry biomass cost information, and provide a tool for future use in analyzing biomass production costs and comparing production methods at other sites.

Switchgrass water use efficiency. Agricultural use of water has become a great concern in western parts of the Great Plains, especially regarding effects of bioenergy crop production on water quality and quantity. ARS scientists at Mandan, ND compared the water use efficiency and soil water deficits for switchgrass (a bioenergy grass), western wheatgrass and a western

wheatgrass-alfalfa mixture (two common forage crops). Water use efficiency was strongly influenced by biomass production and the high productivity of switchgrass resulted in the highest water use efficiency. The water use efficiency of switchgrass was nearly 4 to 5 times that of western wheatgrass, which had water use efficiency that was much more variable. Although switchgrass had the highest water use efficiency, it also had the greatest soil water deficit. This research suggests that switchgrass is a productive bioenergy crop for the drier areas of the northern Great Plains but its greater depletion of soil water may be an issue in a multi-year drought or if switchgrass is used in annual crop rotation.

Developing more efficient methods to collect round bales in-field for transporting to biomass processing. Often biomass logistics are considered to be a rather simple point-to-point transportation of material. However, often biomass is harvested in large round bales which are dispersed over a large area. Therefore, a series of different bale layouts and equipment were evaluated to determine the most efficient way to aggregate bales for transport. Field shape, swath width, biomass yield and randomness of bale layout did not impact aggregation logistics but area and number of bales handled had significant impacts. Use of additional equipment and loaders handling more bales increased efficiency. A self-loading bale picker was the highest ranked method while a central grouping of bales was the lowest ranked method.

Collaborative research helps identify promising technologies to process biofuel feedstocks. Perennial grasses and corn stalks can supply abundant lignocellulosic feedstock in the northern Great Plains of the U.S. There is a need to understand the mechanical properties of these crops for better handling and processing of biomass feedstocks in bioprocessing industries. Ultimate shear stresses were not statistically different for big bluestem, corn stalk, and intermediate wheatgrass, with values of 7.33, 8.53 and 6.23 MPa, respectively, which were less than switchgrass at 13.39 MPa ($p < 0.05$). Corn stalk had the greatest ultimate tensile stress of 69.30 MPa, followed by switchgrass, big bluestem, and intermediate wheatgrass. Based on these results, shear-dominant size-reduction devices (e.g., knife mills and chippers) should be more energy efficient, and this energy efficiency should be taken advantage of when designing size-reduction devices.

Use of digital image analysis to identify different stem components of biomass. Different stem components, such as nodes and internodes, of perennial biomass feedstocks have different chemical composition. Therefore, separating them into ‘segregated processing’ channels could lead to better handling, more efficient processing and higher-value products generation. Differences between nodes and internodes can be visually identified so a digital image analysis should be possible. A MATLAB algorithm was developed to help identify nodes and internodes in chopped stems of big bluestem, switchgrass and corn stalks. A best feature was identified that could identify nodes and internodes with a 99.9% accuracy. This image processing method can be supporting software for a hardware system that would separate nodes and internodes into different processing channels.

Funded Grants:

1. 2012. “Accelerated Development of Commercial Hydrotreated Renewable Jet (HRJ) Fuel from Redesigned Oil Seed Feedstock Supply Chains”. USDA-NIFA Biomass Research and Development Initiative. Award \$6,700,862. PI Terry Isbell, Award to NGPRL: \$362,565.
2. 2011. “Resource assessment framework for dependable feedstock supply to produce advance biofuels in Hawaii and western United States”. U.S. Navy-Office of Naval Research. Award \$6,000,000. Award to NGPRL: \$289,000.
3. 2011. “Improving production, resilience, and biodiversity of perennial grass mixtures and monocultures as biofuel feedstocks”. NC Sun Grant Center. Award \$ \$806,484. PI W. Carter Johnson, Award to NGPRL: \$35,718.

Selected Publications (2010-2014)

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